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(54) Title: TARGETABLE VECTOR PARTICLES

(57) Abstract

A vector particle (e.g., a retroviral vector particle) containing a chimeric envelope includes a receptor binding region that binds to a receptor of a target cell. The receptor of the target cell is other than the amphotropic cell receptor. The receptor binding region may be a receptor binding region of a human virus. A portion of the envelope gene may be deleted and the deleted portion is replaced with another receptor binding region or ligand. Such vector particles are targetable to a desired target cell or tissue, and may be administered directly to the desired target cell or tissue as part of a gene therapy procedure, or administered directly into the patient.

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TARGETABLE VECTOR PARTICLES

This invention relates to "targetable" vector particles. More particularly, this invention relates to vector particles which include a receptor binding region that binds to a receptor of a target cell of a human or non-human animal.

Vector particles are useful agents for introducing gene(s) or DNA (RNA) into a cell, such as a eukaryotic cell. The gene(s) is controlled by an appropriate promoter. Examples of vectors which may be employed to generate vector particles include prokaryotic vectors, such as bacterial vectors; eukaryotic vectors, including fungal vectors such as yeast vectors; and viral vectors such as DNA virus vectors, RNA virus vectors, and retroviral vectors. Retroviral vectors which have been employed for generating vector particles for introducing genes or DNA (RNA) into a cell include Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus and Harvey Sarcoma Virus. The term "introducing" as used herein encompasses a variety of methods of transferring genes or DNA (RNA) into a cell, such methods including transformation, transduction, transfection, and infection.

Vector particles have been used for introducing DNA (RNA) into cells for gene therapy purposes. In general, such a procedure involves obtaining cells from a patient and using a vector particle to introduce desired DNA (RNA) into the cells and

then providing the patient with the engineered cells for a therapeutic purpose. It would be desirable to provide alternative procedures for gene therapy. Such an alternative procedure would involve genetically engineering cells in vivo. In such a procedure, a vector particle which includes the desired DNA (RNA) would be administered directly to the target cells of a patient in vivo.

It is therefore an object of the present invention to provide gene therapy by introduction of a vector particle, such as, for example, a retroviral vector particle, directly into a desired target cell of a patient.

In accordance with an aspect of the present invention, there is provided a retroviral vector particle which includes a receptor binding region or ligand that binds to a receptor of a target cell. The receptor of the target cell is a receptor other than the amphotropic cell receptor.

Retroviruses have an envelope protein which contains a receptor binding region. Applicants have found that retroviruses can be made "targetable" to a specific type of cell if the receptor binding region of the retrovirus, which may be amphotropic, ecotropic, or xenotropic, among other types, is modified such that the receptor binding region of the envelope protein includes a receptor binding region which binds to a receptor of a target cell. For example, at least a portion of the receptor binding region of the envelope protein of the retrovirus is deleted and replaced with a receptor binding region or a ligand which binds to a receptor of a target cell. Thus, there is provided a retroviral vector wherein at least a portion of the DNA (RNA) which encodes the receptor binding region of the envelope protein of the retrovirus has been deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

In one embodiment, the retrovirus is a murine leukemia virus.

The envelope of murine leukemia viruses includes a protein known as gp70. Such viruses can be made "targetable" to a specific type of cell if a portion of the gp70 protein is deleted and replaced with a receptor binding region or a ligand which binds to a receptor of a target cell. Thus, in a preferred embodiment, there is provided a retroviral vector wherein a portion, but not all, of the DNA (RNA) encoding gp70 protein has been deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

In general, gp70 protein includes the following regions: (i) the secretory signal or "leader" sequence; (ii) the receptor binding domain; (iii) the hinge region; and (iv) the body portion. Preferably, at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding the entire receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. In another embodiment, DNA (RNA) encoding the entire receptor binding domain of gp70 protein, plus all or a portion of the DNA (RNA) encoding the hinge region of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand of a target cell.

The gp70 protein may be derived from an ecotropic murine leukemia virus, a xenotropic murine leukemia virus, or an amphotropic murine leukemia virus. Ecotropic gp70 (or eco gp70) (SEQ ID NO:1) is a protein having 469 amino acids, and is encoded by (SEQ ID:2). Amino acid residues 1-33 constitute the leader sequence; amino acid residues 34-263 constitute the receptor binding domain; amino acid residues 264-312 constitute the hinge region; and amino acid residues 313-469 constitute the body portion. Preferably, DNA (RNA) encoding at least a portion of

the receptor binding region is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding some or all of amino acid residues 34 to 263 (i.e., the receptor binding domain) is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

Xenotropic gp70 (or xeno gp70) (SEQ ID NO:3) has 443 amino acid residues and is encoded by (SEQ ID NO:4). Amino acid residues 1-30 constitute the leader sequence; amino acid residues 31-232 constitute the receptor binding domain; amino acid residues 233-286 constitute the hinge region; and amino acid residues 287-443 constitute the body portion. Preferably, DNA (RNA) encoding at least a portion of the receptor binding region is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding some or all of amino acid residues 31 to 232 is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

Target cells to which the retroviral vector particle may bind include, but are not limited to, liver cells, T-cells, lymphocytes, endothelial cells, T4 helper cells, and macrophages. In one embodiment, the retroviral vector particle binds to a liver cell, and in particular to hepatocytes. To enable such binding, the retroviral vector particle contains a chimeric protein encoded by DNA (RNA) in which at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is removed and is replaced with DNA (RNA) which encodes a protein which binds to an asialoglycoprotein receptor (or ASG-R) of hepatocytes.

Proteins which bind to the asialoglycoprotein receptor of liver cells include, but are not limited to, asialoglycoproteins such as, for example, alpha-1-acid glycoprotein (AGP), also known

as orosomucoid, and asialofetuin. AGP is a natural high-affinity ligand for ASG-R. The asialoglycoprotein receptor, or ASG-R, is expressed only by hepatocytes. The receptor is present at about $3x10^5$ copies per cell, and such receptors have a high affinity for asialoglycoproteins such as AGP. Thus, the engineering of retroviral vector particles to contain asialoglycoprotein in place of the natural receptor binding domain of gp70 generates retroviral vector particles which bind to the asialoglycoprotein receptor of hepatocytes, which provides for an efficient means of transferring genes of interest to liver cells.

Cell lines which generate retroviral vector particles that are capable of targeting the hepatocyte's asialoglycoprotein receptor without the removal of the particle's terminal sialic acid groups by neuraminidase treatment, can be developed by selection with the cytotoxic lectin, wheat germ agglutinin (WGA). Cell lines which express the retroviral proteins gag and pol become retroviral vector packaging cell lines after they are transfected with the plasmids encoding chimeric envelope genes. These cell lines express the corresponding chimeric gp 70 glycoproteins. Upon exposure to successively higher concentrations of WGA, the outgrowth of cells which synthesize glycoproteins that lack terminal sialic acid groups, is favored. (Stanley, et al., Somatic Cell Genetics, Vol. 3, pgs. 391-405 (1977)). This selection permits the isolation of cells which synthesize oligosaccharides terminating in galactosyl sugar groups. Such cells will allow the construction of packaging cell lines that are capable of generating retroviral vector particles which target the asialoglycoprotein receptor. It is also possible to select subpopulations of packaging cells which have other distinct glycotypes, such cells yielding viral vectors that potentially are capable of targeting cells other than hepatocytes. Macrophages, for example, express unique, high-mannose receptors. The PHA-resistant subpopulation will have N-linked oligosaccharides which terminate in high-mannose

groups (Stanley, et al., In Vitro, Vol. 12, pgs. 208-215 (1976)). Therefore, such a cell population will be capable of producing viral vector particles capable of targeting macrophoges via this receptor. Cells with mutant glycotypes which synthesize other novel oligosaccharides after selection with other cytotoxic lectins may also prove to be useful in targeting vector particles to other cell types such as lymphocytes or endothelial cells.

In another embodiment, the receptor binding region is a receptor binding region of a human virus. In one embodiment, the receptor binding region of a human virus is a hepatitis B virus surface protein binding region, and the target cell is a liver cell.

In another embodiment, the receptor binding region of a human virus is the gp46 protein of HTLV-I virus, and the target cell is a T-cell.

In yet another embodiment, the receptor binding region of a human virus is the HIV gp120 CD4 binding region, and the target cell is a T4 helper cell.

In one embodiment, the retroviral vector may be of the LN series of vectors, as described in Bender, et al., J. Virol., Vol. 61, pgs. 1639-1649 (1987), and Miller, et al., Biotechniques, Vol. 7, pgs. 98-990 (1989).

In another embodiment, the retroviral vector includes a multiple restriction enzyme site, or multiple cloning site. The multiple cloning site includes at least four cloning, or restriction enzyme sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; i.e., the restriction product has an average size of at least 10,000 base pairs.

In general, such restriction sites, also sometimes hereinafter referred to as "rare" sites, which have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs, contain a CG doublet within their recognition sequence, such doublet appearing particularly infrequently in the

mammalian genome. Another measure of rarity or scarcity of a restriction enzyme site in mammals is its representation in mammalian viruses, such as SV40. In general, an enzyme whose recognition sequence is absent in SV40 may be a candidate for being a "rare" mammalian cutter.

Examples of restriction enzyme sites having an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs include, but are not limited to the NotI, SnaBI, SalI, XhoI, ClaI, SacI, EagI, and SmaI sites. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI.

Preferably, the multiple cloning site has a length no greater than about 70 base pairs, and preferably no greater than about 60 base pairs. In general, the multiple restriction enzyme site, or multiple cloning site is located between the 5' LTR and 3' LTR of the retroviral vector. The 5' end of the multiple cloning site is no greater than about 895 base pairs from the 3' end of the 5' LTR, preferably at least about 375 base pairs from the 3' end of the 5' LTR. The 3' end of the multiple cloning site is no greater than about 40 base pairs from the 5' end of the 3' LTR, and preferably at least 11 base pairs from the 5' end of the 3' LTR.

Such vectors may be engineered from existing retroviral vectors through genetic engineering techniques known in the art such that the retroviral vector includes at least four cloning sites wherein at least two of the cloning sites are selected from the group consisting of the NotI, SnaBI, SalI, and XhoI cloning sites. In a preferred embodiment, the retroviral vector includes each of the NotI, SnaBI, SalI, and XhoI cloning sites.

Such a retroviral vector may serve as part of a cloning system for the transfer of genes to such retroviral vector. Thus, there may be provided a cloning system for the manipulation of genes in a retroviral vector which includes a retroviral vector including a multiple cloning site of the type hereinabove

described, and a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral vector. The shuttle cloning vector also includes at least one desired gene which is capable of being transferred from said shuttle cloning vector to said retroviral vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible, or complementary cloning sites hereinabove described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art.

The shuttle cloning vector may be employed to amplify DNA sequences in prokaryotic systems. The shuttle cloning vector may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322; pUC18; etc.

Such retroviral vectors are transfected or transduced into a packaging cell line, whereby there are generated infectious vector particles which include the retroviral vector. In general, the vector is transfected into the packaging cell line along with a packaging defective helper virus which includes genes encoding the gag and pol, and the env proteins of the virus. Representative examples of packaging cell lines include, but are not limited to, the PE501 and PA317 cell lines disclosed in Miller, et al., Biotechniques, Vol. 7 pgs. 980-990 (1989).

The vector particles generated from the packaging cell line, which are also engineered with a protein containing a receptor binding region that binds to a receptor of a target cell, said receptor being other than the amphotropic cell receptor, are

targetable, whereby the receptor binding region enables the vector particles to bind to a target cell.. The retroviral vector particles thus may be directly administered to a desired target cell ex vivo, and such cells may then be administered to a patient as part of a gene therapy procedure.

Although the vector particles may be administered directly to a target cell, the vector particles may be engineered such that the vector particles are "injectable" as well as targetable; i.e., the vector particles are resistant to inactivation by human serum, and thus the targetable vector particles may be administered to a patient by intravenous injection, and travel directly to a desired target cell or tissue without being inactivated by human serum.

The envelope of retroviruses also includes a protein known as p15E, and Applicants have found that retroviruses are susceptible to inactivation by human serum a a result of the action of complement protein(s) present in serum on the p15E protein portion of the retrovirus. Applicants have further found that such retroviruses can be made resistant to inactivation by human serum by mutating such p15E protein.

In one embodiment, therefore, the retroviral vector is engineered such that a portion of the DNA (RNA) encoding p15E protein (shown in the accompanying sequence listing as SEQ ID NO:7), has been mutated to render the vector particle resistant to inactivation by human serum; i.e., at least one amino acid but not all of the amino acids of the p15E protein has been changed, or mutated.

p15E protein is a viral protein having 196 amino acid residues. In viruses, sometimes all 196 amino acid residues are present, and in other viruses, amino acid residues 181 to 196 (known as the "r" peptide), are not present, and the resulting protein is the "mature" form of p15E known as p12E. Thus, viruses can contain both the p15E and p12E proteins. p15E protein is anchored in the viral membrane such that amino acid

residues residues 1 to 134 are present on the outside of the virus. Although this embodiment of the present invention is not to be limited to any of the following reasoning, Applicants believe complement proteins may bind to this region whereby such binding leads to inactivation and/or lysis of the retrovirus. In particular, the p15E protein includes two regions, amino acid residues 39 to 61 (sometimes hereinafter referred to as region 1), and amino acid residues 101 to 123 (sometimes hereinafter referred to as region 2), which Applicants believe have an external location in the three-dimensional structure of the p15E protein; i.e., such regions are directly exposed to human serum. Region 2 is a highly conserved region in many retroviruses, even though the amino acid sequences of this region are not identical in all retroviruses. Such regions are complement binding regions. Examples of complement proteins which may bind to the complement binding regions are C1S and C1Q, which bind to regions 1 and 2.

In order to inactivate the retrovirus, complement proteins bind to both region 1 and region 2. Thus, in a preferred embodiment, at least one portion of DNA encoding a complement binding region of p15E protein has been mutated. Such a mutation results in a change of at least one amino acid residue of a complement binding region of p15E protein. The change in at least one amino acid residue of a complement binding region of p15E protein prevents binding of a complement protein to the complement binding region, thereby preventing complement inactivation of the retrovirus. In one embodiment, at least one amino acid residue in both complement binding regions of p15E protein is changed, whereas in another embodiment, at least one amino acid residue in one of the complement binding regions is changed.

It is to be understood, however, that the entire DNA sequence encoding p15E protein cannot be mutated because such a change renders the vectors unsuitable for <u>in vivo</u> use.

In one embodiment, the mutation of DNA (RNA) encoding p15E protein may be effected by deleting a portion of the p15E gene, and replacing the deleted portion of the p15E gene, with fragment(s) or portion(s) of a gene encoding another viral protein. In one embodiment, one portion of DNA encoding the p15E protein is replaced with a fragment of the gene encoding the p21 protein, which is an HTLV-I transmembrane protein. HTLV-I virus has been found to be resistant to binding by complement proteins and thus HTLV-I is resistant to inactivation by human serum (Hoshino, et al., Nature, Vol. 310, pgs. 324-325 (1984)). Thus, in one embodiment, there is also provided a retroviral vector particle wherein a portion of the p15E protein has been deleted and replaced with a portion of another viral protein, such as a portion of the p21 protein.

p21 protein (as shown in the accompanying sequence listing as SEQ ID NO:8) is a protein having 176 amino acid residues, and which, in relation to p15E, has significant amino acid sequence homology. In one embodiment, at least amino acid residues 39 to 61, and 101 to 123 are deleted from p15E protein, and replaced with amino acid residues 34 to 56 and 96 to 118 of p21 protein. In one alternative, at least amino acid residues 39 to 123 of p15E protein are deleted and replaced with amino acid residues 34 to 118 of p21 protein.

In another embodiment, amino acid residues 39 to 69 of p15E protein are deleted and replaced with amino acid residues 34 to 64 of p21 protein, and amino acid residues 96 to 123 of p15E protein are deleted and replaced with amino acid residues 91 to 118 of p21 protein.

Vector particles generated from such packaging lines, therefore, are "targetable" and "injectable," whereby such vector particles, upon administration to a patient, travel directly to a desired target cell or tissue.

The targetable vector particles are useful for the introduction of desired heterologous genes into target cells ex

vivo. Such cells may then be administered to a patient as a gene therapy procedure, whereas vector particles which are targetable and injectable may be administered in vivo to the patient, whereby the vector particles travel directly to a desired target cell.

Thus, preferably, the vectors or vector particles of the present invention further include at least one heterologous gene. Heterologous or foreign genes which may be placed into the vector or vector particles include, but are not limited to, genes which encode cytokines or cellular growth factors, such as lymphokines, which are growth factors for lymphocytes. Other examples of foreign genes include, but are not limited to, genes encoding Factor VIII, Factor IX, tumor necrosis factors (TNF's), ADA, ApoE, ApoC, and Protein C.

The vectors of the present invention include one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., Biotechniques, Vol. 7, No. 9, pgs 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and B-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The vectors of the present invention may contain regulatory elements, where necessary to ensure tissue specific expression of the desired heterologous gene(s), and/or to regulate expression of the heterologous gene(s) in response to cellular or metabolic signals.

Although the invention has been described with respect to retroviral vector particles, other viral vector particles (such as, for example, adenovirus, adeno-associated virus, and Herpes

Simplex virus particles), or synthetic particles may be constructed such that the vector particles include a receptor binding region that binds to a receptor of a target cell, wherein the receptor of a human target cell is other than the amphotropic cell receptor. Such vector particles are suitable for in vivo administration to a desired target cell.

Advantages of the present invention include the ability to provide vector particles which may be administered directly to a desired target cell or tissues, whereby desired genes are delivered to the target cell or tissue, whereby the target cell or tissue may produce the proteins expressed by such genes.

This invention will now be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Plasmid pCee (Figure 1), which contains the ecotropic murine leukemia virus gp70 and p15E genes under the control of a CMV promoter, was cut with AccI, and an AccI fragment encoding amino acid residues 1-312 of the eco gp70 protein was removed. Cloned into the AccI site was a PCR fragment containing the eco gp70 secretion signal (or leader, which includes amino acid residues 1-33 of eco gp70), followed by mature rabbit alpha-1 acid glycoprotein (amino acid residues 19-201) (Ray, et al., Biochemical and Biophysical Research Communications, Vol. 178, No. 2, pgs. 507-513 (1991)). The amino acid sequence of rabbit alpha-1 acid glycoprotein is shown in (SEQ ID NO:5), and the DNA sequence encoding therefor is shown in (SEQ ID NO:6). The resulting plasmid pAGP-1 (Figure 2) contains the eco gp70 leader sequence (amino acid residues 1-33 of eco gp70), a sequence encoding the mature rabbit alpha-1 acid glycoprotein (amino acid residues 19-201), and a sequence encoding amino acid residues 313 to 469 of eco gp70.

Example 2

Plasmid pC e was cut with SalI and PflMI, and a SalI-PflMI fragment encoding amino acid residues 1-262 of eco gp70 was removed. Cloned into this site was a PCR generated SalI-PflMI fragment containing the eco gp70 leader sequence and the sequence encoding mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAGP-3 (Figure 3) thus includes a sequence encoding the leader sequence of eco gp70, a sequence encoding mature rabbit alpha-1 acid glycoprotein; and a sequence encoding amino acid residues 263 to 469 of eco gp70.

Example 3

Plasmid pUC18RSVXeno (Figure 4), which contains the xenotrophic murine leukemia virus gp70 and p15E genes under the control of an RSV promoter, was cut with AccI and StuI, and an AccI-StuI fragment encoding amino acid residues 1-258 of xeno gp70 was removed. Cloned into this site was a PCR generated AccI-StuI fragment encoding the xeno gp70 leader (amino acid residues 1-30), and the mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAX2 (Figure 5), thus contains a sequence encoding the xeno gp70 leader, a sequence encoding the mature rabbit alpha-1 acid glycoprotein, and amino acid residues 259-443 of xeno gp70.

Example 4

Plasmid pUC18RSVXeno was cut with AccI and ClaI, and a fragment encoding amino acid residues 1-210 of xeno gp70 was removed. Cloned into this site was a PCR generated AccI-ClaI fragment encoding the xeno gp70 leader, followed by mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAX6 (Figure 6), thus includes a sequence encoding the xeno gp70 leader, a sequence encoding mature rabbit alpha-1 acid glycoprotein, and amino acid residues 211-443 of xeno gp70.

Example 5

$5x10^5$ GPL cells on 10 cm tissue culture plates were transfected (using $CaPO_4$) with 30 µg/plate of one of plasmids pAGP-1, pAGP-3, pAX2, or pAX6. The $CaPO_4$ is removed 24 hours later and 10 ml of fresh D10 medium is added for another 24 hours. The D10 medium is then removed and replaced with serum free DX medium for another 24 hours. The DX medium is then collected, filtered, and stored on ice. This supernatant contains the vector particles.

The supernatants were then filtered and collected by standard procedures and then centrifuged. After centrifugation, the virus pellets were reconstituted in a buffer containing 0.1M sodium acetate, 0.15M sodium chloride, and 2mM calcium chloride;

the buffer was sterilized using a Falcon 0.2 millimicron tissue culture filter.

2.2 ml of concentrated supernatant containing viral particles generated from pAGP-1 or pAGP-3, said viral particles sometimes hereinafter referred to as Chimeric-1 or Chimeric-3, were loaded onto two disposable plastic columns which were alcohol sterilized and dried. To each column (1cm x 6cm), one unit of neuraminidase from Clostridium perfringens which was bound to beaded agarose was added as a 2 ml suspension. This represents 1 ml of packed gel or unit of enzyme per column (15.7 mg of agarose/ml and 28 units per gram of agarose). A unit is defined as the amount of neuraminidase which will liberate 1.0 micromole of N-acetylneuraminic acid per minute from NAN-lactose at pH 5.0 and 37°C.

The columns were then washed with a large excess (50 ml) of the buffer hereinabove described to free the resin of all traces of free neuraminidase and to sterilize the resin prior to incubation with virus. The columns were then dried, and the bottoms were sealed with caps and secured with parafilm. The concentrated virus which was reconstituted in the buffer (2.0 ml per sample) was then added to the resin. The tops were placed on the columns and secured with parafilm. The resin was gently re-suspended by hand. The virus was then incubated with the resin for 1 hour at room temperature with gentle rotation on a wheel. The columns were checked periodically to ensure good mixing of resin and virus.

At the end of the incubation period, the Chimera-1 and Chimera-3 viruses were recovered by gentle vacuum filtration and collected into separate sterile 12x75 mm plastic polypropylene Falcon 2063 tubes. Recovery was greater than 90%, giving about 1.8 ml of desialated virus.

6-well plates containing about 10^5 receptor-positive (Hep G2) or receptor-negative (SK HepI) human hepatocytes in 2 ml D10 media were employed as target cells. 24 hours after the cells

were plated, 1 ml of D10 was removed from the first well and 2 ml of neuraminidase-treated (or untreated as a control) viral supernatant containing Chimeric-1 or Chimeric-3 was added and mixed well. 200 ul from the first well was diluted into the 2 ml present in the second well, was then mixed; and then 200 ul from the second well was diluted into the 1.8 ml present in the third well, thereby giving approximate dilutions of 2/3, 1/15, and 1/150. 8 ug/ml of Polybrene was included in each well during the transduction. The viral particles were left in contact with the cells overnight, followed by removal of media containing viral particles, and replaced with D10 containing 1,000 mg/ml of G418. The medium was changed with fresh D10 and G418 every 4 to 5 days as necessary. G418-resistant colonies were scored after 2 to 3 weeks.

Example 6

The pre-packaging cell line GP8, which expresses the retroviral proteins gag and pol, and the packaging cell lines derived from them which also express the chimeric gp70 glycoproteins encoded by the plasmids pAGP-1, pAGP-3, pAX2, or pAX6 were maintained in cell culture and exposed to successively higher concentrations of wheat germ agglutinin; starting with 15 ug/ml. The cell lines were maintained under WGA selection in cell culture for 6 to 8 weeks until populations resistant to 40-50 ug/ml WGA were obtained. The latter were then subjected to fluoresence-activated cell sorting using FITC-conjugated lectins to enrich for the cells expressing the desired mutant glycotype (e.g., FITC-Erythrina Cristagalli agglutinin for beta-D-galactosyl groups, and FITC-concanavalin A for alpha-D-mannosyl groups). Retroviral vector packaging and producer cell lines were then generated from the resulting populations by standard techniques.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced

other than as particularly described and still be within the scope of the accompanying claims.

PATAP697

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Targetable Vector Particles

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(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch diskette
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: DW4.V2

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lillie, Raymond J.
(B) REGISTRATION NUMBER: 31,778
(C) REFERENCE/DOCKET NUMBER: 271010-107

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 469 bases

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: Ecotropic gp70 Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Arg Ser Thr Leu Ser Lys Pro Leu
5 10
Lys Asn Lys Val Asn Pro Arg Gly Pro Leu
15 20
Ile Pro Leu Ile Leu Leu Met Leu Arg Gly
25 30
Val Ser Thr Ala Ser Pro Gly Ser Ser Pro
35 40
His Gly Val Tyr Asn Ile Thr Trp Glu Val
45 50
Thr Asn Gly Asp Arg Glu Thr Val Trp Ala
55 60
Thr Ser Gly Asn His Pro Leu Trp Thr Trp
65 70
Trp Pro Asp Leu Thr Pro Asp Leu Cys Met
75 80
Leu Ala His His Gly Pro Ser Tyr Trp Gly
85 90
Leu Glu Tyr Gln Ser Pro Phe Ser Ser Pro
95 100
Pro Gly Pro Pro Cys Cys Ser Gly Gly Ser

105 110

Ser Pro Gly Cys Ser Arg Asp Cys Glu Glu

115 120

Pro Leu Thr Ser Leu Thr Pro Arg Cys Asn

125 130

Thr Ala Trp Asn Arg Leu Lys Leu Asp Gln

135 140

Thr Thr His Lys Ser Asn Glu Gly Phe Tyr

145 150

Val Cys Pro Gly Pro His Arg Pro Arg Glu

155 160

Ser Lys Ser Cys Gly Gly Pro Asp Ser Phe

165 170

Tyr Cys Ala Tyr Trp Gly Cys Glu Thr Thr

175 180

Gly Arg Ala Tyr Trp Lys Pro Ser Ser Ser

185 190

Trp Asp Phe Ile Thr Val Asn Asn Asn Leu

195 200

Thr Ser Asp Gln Ala Val Gln Val Cys Lys

205 210

Asp Asn Lys Trp Cys Asn Pro Leu Val Ile

215 220

Arg Phe Thr Asp Ala Gly Arg Arg Val Thr

225 230

Ser Trp Thr Thr Gly His Tyr Trp Gly Leu

235 240

Arg Leu Tyr Val Ser Gly Gln Asp Pro Gly

245 250

Leu Thr Phe Gly Ile Arg Leu Arg Tyr Gln

255 260

Asn Leu Gly Pro Arg Val Pro Ile Gly Pro

265 270

Asn Pro Val Leu Ala Asp Gln Gln Pro Leu

275 280
Ser Lys Pro Lys Pro Val Lys Ser Pro Ser
285 290
Val Thr Lys Pro Pro Ser Gly Thr Pro Leu
295 300
Ser Pro Thr Gln Leu Pro Pro Ala Gly Thr
305 310
Glu Asn Arg Leu Leu Asn Leu Val Asp Gly
315 320
Ala Tyr Gln Ala Leu Asn Leu Thr Ser Pro
325 330
Asp Lys Thr Gln Glu Cys Trp Leu Cys Leu
335 340
Val Ala Gly Pro Pro Tyr Tyr Glu Gly Val
345 350
Ala Val Leu Gly Thr Tyr Ser Asn His Thr
355 360
Ser Ala Pro Ala Asn Cys Ser Val Ala Ser
365 370
Gln His Lys Leu Thr Leu Ser Glu Val Thr
375 380
Gly Gln Gly Leu Cys Ile Gly Ala Val Pro
385 390
Lys Thr His Gln Ala Leu Cys Asn Thr Thr
395 400
Gln Thr Ser Ser Arg Gly Ser Tyr Tyr Leu
405 410
Val Ala Pro Thr Gly Thr Met Trp Ala Cys
415 420
Ser Thr Gly Leu Thr Pro Cys Ile Ser Thr
425 430
Thr Ile Leu Asn Leu Thr Thr Asp Tyr Cys
435 440
Val Leu Val Glu Leu Trp Pro Arg Val Thr

445 450
Tyr His Ser Pro Ser Tyr Val Tyr Gly Leu
455 460
Phe Glu Arg Ser Asn Arg His Lys Arg
465

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1446 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: viral DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCTGCCGAC CCCGGGGGTG GACCATCCTC TAGACTGACA TGGCGCGTTA AACGCTCTCA 60

AAACCCCTTA AAAATAAGGT TAACCCGCGA GGCCCCCTAA TCCCCTTAAT TCTTCTGATG 120

CTCAGAGGGG TCAGTACTGC TTCGCCCGGC TCCAGTCCTC ATCAAGTCTA TAATATCACC 180

TGGGAGGTAA CCAATGGAGA TCGGGAGACG GTATGGGCAA CTTCTGGCAA CCACCCTCTG 240

TGGACCTGGT GGCCTGACCT TACCCCAGAT TTATGTATGT TAGCCCACCA TGGACCATCT 300

TATTGGGGGC TAGAATATCA ATCCCCTTTT TCTTCTCCCC CGGGGCCCCC TTGTTGCTCA 360

GGGGGCAGCA GCCCAGGCTG TTCCAGAGAC TGCGAAGAAC CTTTAACCTC CCTCACCCCT 420

CGGTGCAACA CTGCCTGGAA CAGACTCAAG CTAGACCAGA CAACTCATAA ATCAAATGAG 480

GGATTTTATG TTTGCCCCGG GCCCCACCGC CCCCGAGAAT CCAAGTCATG TGGGGGTCCA 540

GACTCCTTCT ACTGTGCCTA TTGGGGCTGT GAGACAACCG GTAGAGCTTA CTGGAAGCCC 600

TCCTCATCAT GGGATTTCAT CACAGTAAAC AACAATCTCA CCTCTGACCA GGCTGTCCAG 660

GTATGCAAAG ATAATAAGTG GTGCAACCCC TTAGTTATTC GGTTTACAGA CGCCGGGAGA 720

CGGGTTACTT CCTGGACCAC AGGACATTAC TGGGGCTTAC GTTTGTATGT CTCCGGACAA 780

GATCCAGGGC TTACATTTGG GATCCGACTC AGATACCAAA ATCTAGGACC CCGCGTCCCA 840

ATAGGGCCAA ACCCCGTTCT GGCAGACCAA CAGCCACTCT CCAAGCCCAA ACCTGTTAAG 900

TCGCCTTCAG TCACCAAACC ACCCAGTGGG ACTCCTCTCT CCCCTACCCA ACTTCCACCG 960

GCGGGAACGG AAAATAGGCT GCTAAACTTA GTAGACGGAG CCTACCAAGC CCTCAACCTC 1020

ACCAGTCCTG ACAAAACCCA AGAGTGCTGG TTGTGTCTAG TAGCGGGACC CCCCTACTAC 1080

GAAGGGGTTG CCGTCCTGGG TACCTACTCC AACCATACCT CTGCTCCAGC CAACTGCTCC 1140

GTGGCCTCCC AACACAAGTT GACCCTGTCC GAAGTGACCG GACAGGGACT CTGCATAGGA 1200

GCAGTTCCCA AAACACATCA GGCCCTATGT AATACCACCC AGACAAGCAG TCGAGGGTCC 1260

TATTATCTAG TTGCCCCTAC AGGTACCATG TGGGCTTGTA GTACCGGGCT TACTCCATGC 1320

ATCTCCACCA CCATACTGAA CCTTACCACT GATTATTGTG TTCTTGTCGA ACTCTGGCCA 1380

AGAGTCACCT ATCATTCCCC CAGCTATGTT TACGGCCTGT TTGAGAGATC CAACCGACAC 1440

AAAAGA 1446

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 443 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY:xenotropic gp70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Glu Gly Ser Ala  Phe Ser Lys Pro Leu
                5                   10
Lys Asp Lys Ile Asn  Pro Trp Gly Pro Leu
               15                   20
Ile Val Met Gly Ile  Leu Val Arg Ala Gly
               25                   30
Ala Ser Val Gln Arg  Asp Ser Pro His Gln
               35                   40
Ile Phe Asn Val Thr  Trp Arg Val Thr Asn
               45                   50
Leu Met Thr Gly Gln  Thr Ala Asn Ala Thr
               55                   60
Ser Leu Leu Gly Thr  Met Thr Asp Thr Phe
               65                   70
```

Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu
75 80

Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu
75 80

Val Gly Asp Tyr Trp Asp Asp Pro Glu Pro
85 90

Asp Ile Gly Asp Gly Cys Arg Thr Pro Gly
95 100

Gly Arg Arg Arg Thr Arg Leu Tyr Asp Phe
105 110

Tyr Val Cys Pro Gly His Thr Val Pro Ile
115 120

Gly Cys Gly Gly Pro Gly Glu Gly Tyr Cys
125 130

Gly Lys Trp Gly Cys Glu Thr Thr Gly Gln
135 140

Ala Tyr Trp Lys Pro Ser Ser Ser Trp Asp
145 150

Leu Ile Ser Leu Lys Arg Gly Asn Thr Pro
155 160

Lys Asp Gln Gly Pro Cys Tyr Asp Ser Ser
165 170

Val Ser Ser Gly Val Gln Gly Ala Thr Pro
175 180

Gly Gly Arg Cys Asn Pro Leu Val Leu Glu
185 190

Phe Thr Asp Ala Gly Arg Lys Ala Ser Trp
195 200

Asp Ala Pro Lys Val Trp Gly Leu Arg Leu
205 210

Tyr Arg Ser Thr Gly Ala Asp Pro Val Thr
215 220

Arg Phe Ser Leu Thr Arg Gln Val Leu Asn
225 230

Val Gly Pro Arg Val Pro Ile Gly Pro Asn
235 240

Pro Val Ile Thr Asp Gln Leu Pro Pro Ser
245 250

Gln Pro Val Gln Ile Met Leu Pro Arg Pro
255 260

Pro His Pro Pro Pro Ser Gly Thr Val Ser
265 270

Met Val Pro Gly Ala Pro Pro Pro Ser Gln
275 280

Gln Pro Gly Thr Gly Asp Arg Leu Leu Asn
285 290

Leu Val Glu Gly Ala Tyr Gln Ala Leu Asn
295 300

Leu Thr Ser Pro Asp Lys Thr Gln Glu Cys
305 310

Trp Leu Cys Leu Val Ser Gly Pro Pro Tyr
315 320

Tyr Glu Gly Val Ala Val Leu Gly Thr Tyr
325 330
Ser Asn His Thr Ser Ala Pro Ala Asn Cys
335 340
Ser Val Ala Ser Gln His Lys Leu Thr Leu
345 350
Ser Glu Val Thr Gly Gln Gly Leu Cys Val
355 360
Gly Ala Val Pro Lys Thr His Gln Ala Leu
365 370
Cys Asn Thr Thr Gln Lys Thr Ser Asp Gly
375 380
Ser Tyr Tyr Leu Ala Ala Pro Ala Gly Thr
385 390
Ile Trp Ala Cys Asn Thr Gly Leu Thr Pro
395 400
Cys Leu Ser Thr Thr Val Leu Asn Leu Thr
405 410
Thr Asp Tyr Cys Val Leu Val Glu Leu Trp
415 420
Pro Lys Val Thr Tyr His Ser Pro Asp Tyr
425 430
Val Tyr Gly Gln Phe Glu Lys Lys Thr Lys
435 440
Tyr Lys Arg

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1356 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: viral DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGACAACTC CTCCAGCCGG GAACAGCATG GAAGGTTCAG CGTTCTCAAA ACCCCTTAAA 60

GATAAGATTA ACCCGTGGGG CCCCCTAATA GTTATGGGGA TCTTGGTGAG GGCAGGAGCT 120

TCGGTACAAC GTGACAGCCC TCACCAGATC TTCAATGTTA CTTGGAGAGT TACCAACCTA 180

ATGACAGGAC AAACAGCTAA CGCCACCTCC CTCCTGGGGA CGATGACAGA CACCTTCCCT 240

AAACTATATT TTGACCTGTG TGATTTAGTA GGAGACTACT GGGATGACCC AGAACCCGAT 300

ATTGGGGATG GTTGCCGCAC TCCCGGGGGA AGAAGAAGGA CAAGACTGTA TGACTTCTAT 360

GTTTGCCCCG GTCATACTGT ACCAATAGGG TGTGGAGGGC CGGGAGAGGG CTACTGTGGC 420

AAATGGGGAT GTGAGACCAC TGGACAGGCA TACTGGAAGC CATCATCATC ATGGGACCTA 480

ATTTCCCTTA AGCGAGGAAA CACTCCTAAG GATCAGGGCC CCTGTTATGA TTCCTCGGTC 540

TCCAGTGGCG TCCAGGGTGC CACACCGGGG GGTCGATGCA ACCCCCTGGT CTTAGAATTC 600

ACTGACGCGG GTAGAAAGGC CAGCTGGGAT GCCCCCAAAG TTTGGGGACT AAGACTCTAT 660

CGATCCACAG GGGCCGACCC GGTGACCCGG TTCTCTTTGA CCCGCCAGGT CCTCAATGTA 720

GGACCCCGCG TCCCCATTGG GCCTAATCCC GTGATCACTG ACCAGCTACC CCCATCCCAA 780

CCCGTGCAGA TCATGCTCCC CAGGCCTCCT CATCCTCCTC CTTCAGGCAC GGTCTCTATG 840

GTACCTGGGG CTCCCCCGCC TTCTCAACAA CCTGGGACGG GAGACAGGCT GCTAAATCTG 900

GTAGAAGGAG CCTACCAAGC ACTCAACCTC ACCAGTCCTG ACAAAACCCA AGAGTGCTGG 960

TTGTGTCTGG TATCGGGACC CCCCTACTAC GAAGGGCTTG CCGTCCTAGG TACCTACTCC 1020

AACCATACCT CTGCCCCAGC TAACTGCTCC GTGGCCTCCC AACACAAGCT GACCCTGTCC 1080

GAAGTAACCG GACAGGGACT CTGCGTAGGA GCAGTTCCCA AAACCCATCA GGCCCTGTGT 1140

AATACCACCC AGAAGACGAG CGACGGGTCC TACTATCTGG CTGCTCCCGC CGGGACCATC 1200

TGGGCTTGCA ACACCGGGCT CACTCCCTGC CTATCTACTA CTGTACTCAA CCTCACCACC 1260

GATTACTGTG TCCTGGTTGA GCTCTGGCCA AAGGTAACCT ACCACTCCCC TGATTATGTT 1320

TATGGCCAGT TTGAAAAGAA AACTAAATAT AAAAGA 1356

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 201 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE

(A) NAME/KEY:rabbit alpha-1-acid glycoprotein

(x) PUBLICATION INFORMATION

(A) AUTHOR Ray, et al.

(B) TITLE:

(C) JOURNAL: Biochem. and Biophys. Res. Comm.

(D) VOLUME: 178

(E) ISSUE: No. 2

(F) PAGES: 507-513

(G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Ala Leu Pro Trp  Ala Leu Ala Val Leu
                5                   10
Ser Leu Leu Pro Leu  Leu His Ala Gln Asp
               15                   20
Pro Ala Cys Ala Asn  Phe Ser Thr Ser Pro
               25                   30
```

Ile Thr Asn Ala Thr Leu Asp Gln Leu Ser
35 40
His Lys Trp Phe Phe Thr Ala Ser Ala Phe
45 50
Arg Asn Pro Lys Tyr Lys Gln Leu Val Gln
55 60
His Thr Gln Ala Ala Phe Phe Tyr Phe Thr
65 70
Ala Ile Lys Glu Glu Asp Thr Leu Leu Leu
75 80
Arg Glu Tyr Ile Thr Thr Asn Asn Thr Cys
85 90
Phe Tyr Asn Ser Ser Ile Val Arg Val Gln
95 100
Arg Glu Asn Gly Thr Leu Ser Lys His Asp
105 110
Gly Ile Arg Asn Ser Val Ala Asp Leu Leu
115 120
Leu Leu Arg Asp Pro Gly Ser Phe Leu Leu
125 130
Val Phe Phe Ala Gly Lys Glu Gln Asp Lys
135 140
Gly Met Ser Leu Tyr Thr Asp Lys Pro Lys
145 150
Ala Ser Thr Glu Gln Leu Glu Glu Phe Tyr
155 160

Glu Ala Leu Thr Cys Leu Gly Met Asn Lys
165 170
Thr Glu Val Val Tyr Thr Asp Trp Thr Lys
175 180
Asp Leu Cys Glu Pro Leu Glu Lys Gln His
185 190
Glu Glu Glu Arg Lys Lys Glu Lys Ala Glu
195 200
Ser

(2) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 759 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(x) PUBLICATION INFORMATION:

(A) AUTHOR Ray, et al.

(B) TITLE:

(C) JOURNAL: Biochem. and Biophys. Res. Comm.

(D) VOLUME: 178

(E) ISSUE: NO. 2

(F) PAGES: 507-513

(G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCTCTGCCT GGCTCCAGCG CCTCTGTGTC TCAGCATGGC CCTGCCCTGG GCCCTCGCCG 60

TCCTGAGCCT CCTCCCTCTG CTGCATGCCC AGGACCCAGC GTGTGCCAAC TTCTCGACCA 120

GCCCTATCAC CAATGCCACC CTGGACCAGC TCTCCCACAA GTGGTTTTTT ACCGCCTCGG 180

CCTTCCGGAA CCCCAAGTAC AAGCAGCTGG TGCAGCATAC CCAGGCGGCC TTTTTCTACT 240

TCACCGCCAT CAAAGAGGAG GACACCTTGC TGCTCCGGGA GTACATAACC ACGAACAACA 300

CGTGCTTCTA TAACTGCAGC ATCGTGAGGG TCCAGAGAGA GAATGGGACC CTCTCCAAAC 360

ACGACGGCAT ACGAAATAGC GTGGCCGACC TGCTGCTCCT CAGGGACCCC GGGAGCTTCC 420

TCCTCGTCTT CTTCGCTGGG AAGGAGCAGG ACAAGGGAAT GTCCTTCTAC ACCGACAAGC 480

CCAAGGCCAG CCCGGAACAA CTGGAAGAGT TCTACGAAGC CCTCACGTGC CTGGGCATGA 540

ACAAGACGGA AGTCGTCTAC ACTGACTGGA CAAAGGATCT GTGCGAGCCG CTGGAGAAGC 600

AACACGAGGA GGAGAGGAAG AAGGAAAAGG CAGAGTCATA GGGCACAGCA CCGGCTCCGG 660

GACTCGGGGC CCACCCCCTG CACCTGCCTT TTTGTTTGTT TTGTAAATCT CTGTTCTTTC 720

CCATGGTTGC ATCAATAAAA CTGCTGGACC AGTAAAAAA 759

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 196 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: ecotropic p15E protein.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Glu Pro Val Ser Leu Thr Leu Ala Leu Leu
5 10

Leu Gly Gly Leu Thr Met Gly Gly Ile Ala
15 20

Ala Gly Ile Gly Thr Gly Thr Thr Ala Leu
25 30

Met Ala Thr Gln Gln Phe Gln Gln Leu Gln
35 40

Ala Ala Val Gln Asp Asp Leu Arg Glu Val
45 50

Glu Lys Ser Ile Ser Asn Leu Glu Lys Ser
55 60
Leu Thr Ser Leu Ser Glu Val Val Leu Gln
65 70
Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu
75 80
Lys Glu Gly Gly Leu Cys Ala Ala Leu Lys
85 90
Glu Glu Cys Cys Phe Tyr Ala Asp His Thr
95 100
Gly Leu Val Arg Asp Ser Met Ala Lys Leu
105 110
Arg Glu Arg Leu Asn Gln Arg Gln Lys Leu
115 120
Phe Glu Ser Thr Gln Gly Trp Phe Glu Gly
125 130
Leu Phe Asn Arg Ser Pro Trp Phe Thr Thr
135 140
Leu Ile Ser Thr Ile Met Gly Pro Leu Ile
145 150
Val Leu Leu Met Ile Leu Leu Phe Gly Pro
155 160
Cys Ile Leu Asn Arg Leu Val Gln Phe Val
165 170
Lys Asp Arg Ile Ser Val Val Gln Ala Leu
175 180

Val Leu Thr Gln Gln Tyr His Gln Leu Lys

185 190

Pro Ile Glu Tyr Glu Pro

195

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 176 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: HTLV-I p21 protein

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Malik, et al.

(B) TITLE:

(C) JOURNAL: J. Gen. Virol.

(D) VOLUME: 69

(E) ISSUE:

(F) PAGES: 1695-1710

(G) DATE: 1988

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Val Pro Val Ala Val Trp Leu Val Ser
5 10

Ala Leu Ala Met Gly Ala Gly Val Ala Gly
15 20

Arg Ile Thr Gly Ser Met Ser Leu Ala Ser
25 30

Gly Lys Ser Leu Leu His Glu Val Asp Lys
35 40

Asp Ile Ser Gln Leu Thr Gln Ala Ile Val
45 50

Lys Asn His Lys Asn Leu Leu Lys Ile Ala
55 60
Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu
65 70
Asp Leu Leu Phe Trp Glu Gln Gly Gly Leu
75 80
Cys Lys Ala Leu Gln Glu Gln Cys Cys Phe
85 90
Leu Asn Ile Thr Asn Ser His Val Ser Ile
95 100
Leu Gln Glu Arg Pro Pro Leu Glu Asn Arg
105 110
Val Leu Thr Gly Trp Gly Leu Asn Trp Asp
115 120
Leu Gly Leu Ser Gln Trp Ala Arg Glu Ala
125 130
Leu Gln Thr Gly Ile Thr Leu Val Ala Leu
135 140
Leu Leu Leu Val Ile Leu Ala Gly Pro Cys
145 150
Ile Leu Arg Gln Leu Arg His Leu Pro Ser
155 160
Arg Val Arg Tyr Pro His Tyr Ser Leu Ile
165 170
Asn Pro Glu Ser Ser Leu
175

WHAT IS CLAIMED IS:

1. A retroviral vect r particle, said vector particle including a receptor binding region that binds to a receptor of a target cell, said receptor of a target cell being other than the amphotropic cell receptor.

2. The vector particle of Claim 1 wherein said vector particle is a murine leukemia virus particle.

3. The vector particle of Claim 2 wherein said vector particle includes gp70 protein, and wherein a portion but not all of the gp70 protein has been deleted and replaced with said receptor binding region that binds to a receptor of a target cell.

4. The vector particle of Claim 1 wherein said receptor binding region is a receptor binding region of a human virus.

5. The vector particle of Claim 4 wherein said receptor binding region of a human virus is a hepatitis B virus surface protein binding region and said target cell is a liver cell.

6. The vector particle of Claim 4 wherein said receptor binding region of a human virus is the receptor binding region of gp46 of HTLV-I virus, and said target cell is a T-cell.

7. The vector particle of Claim 4 wherein said receptor binding region of a human virus is the HIV gp120 CD4 binding region and said target cell is a T4 helper cell.

8. The vector particle of Claim 2 wherein said vector particle contains a chimeric protein encoded by DNA (RNA) wherein at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is removed and is replaced with DNA (RNA) which encodes a protein which binds to an asialoglycoprotein receptor of hepatocytes.

9. The vect r particle of Claim 8 wherein said protein which binds to an asialoglycoprotein recept r of hepatocytes is alpha-1 acid glycoprotein.

10. The vector particle of Claim 1 and further including at least one heterologous gene.

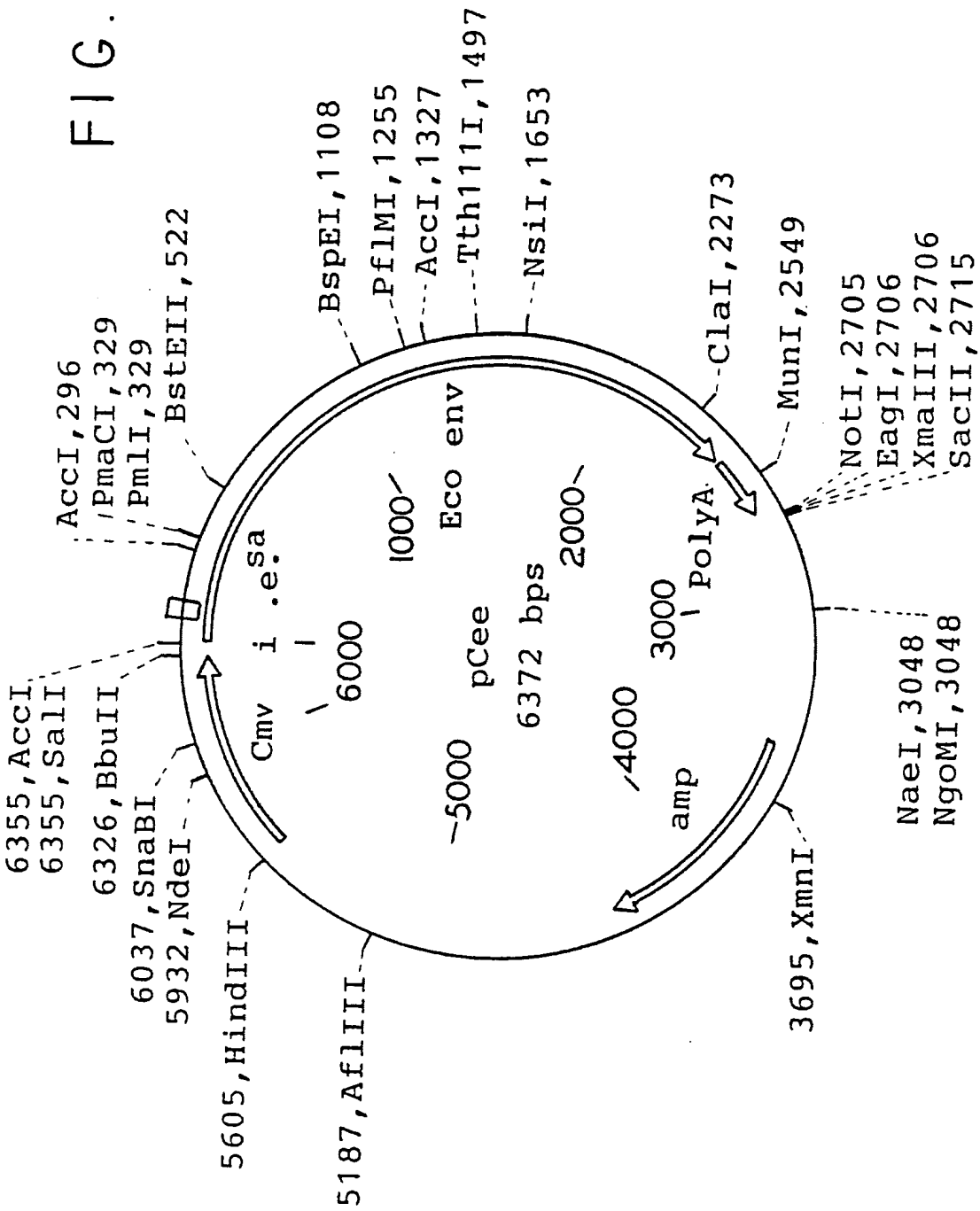
11. A method of introducing at least one heterologous gene into a target cell, comprising, administering to said target cell the vector particles of Claim 10.

12. The method of Claim 11 wherein said vector particles, are administered ex vivo.

13. The method of Claim 11 wherein said vector particles are administered in vivo.

14. A packaging cell line which produces the retroviral particles of Claim 1.

FIG. 1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ClaI,2273
MunI,2549
NotI,2705
EagI,2706
XmaIII,2706
SacII,2715
NaeI,3048
NgoMI,3048
3695,XmnI
5187,AflIII
5605,HindIII
6037,SnaBI
5932,NdeI
6326,BbuII
6355,AccI
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Eco env
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amp
PolyA
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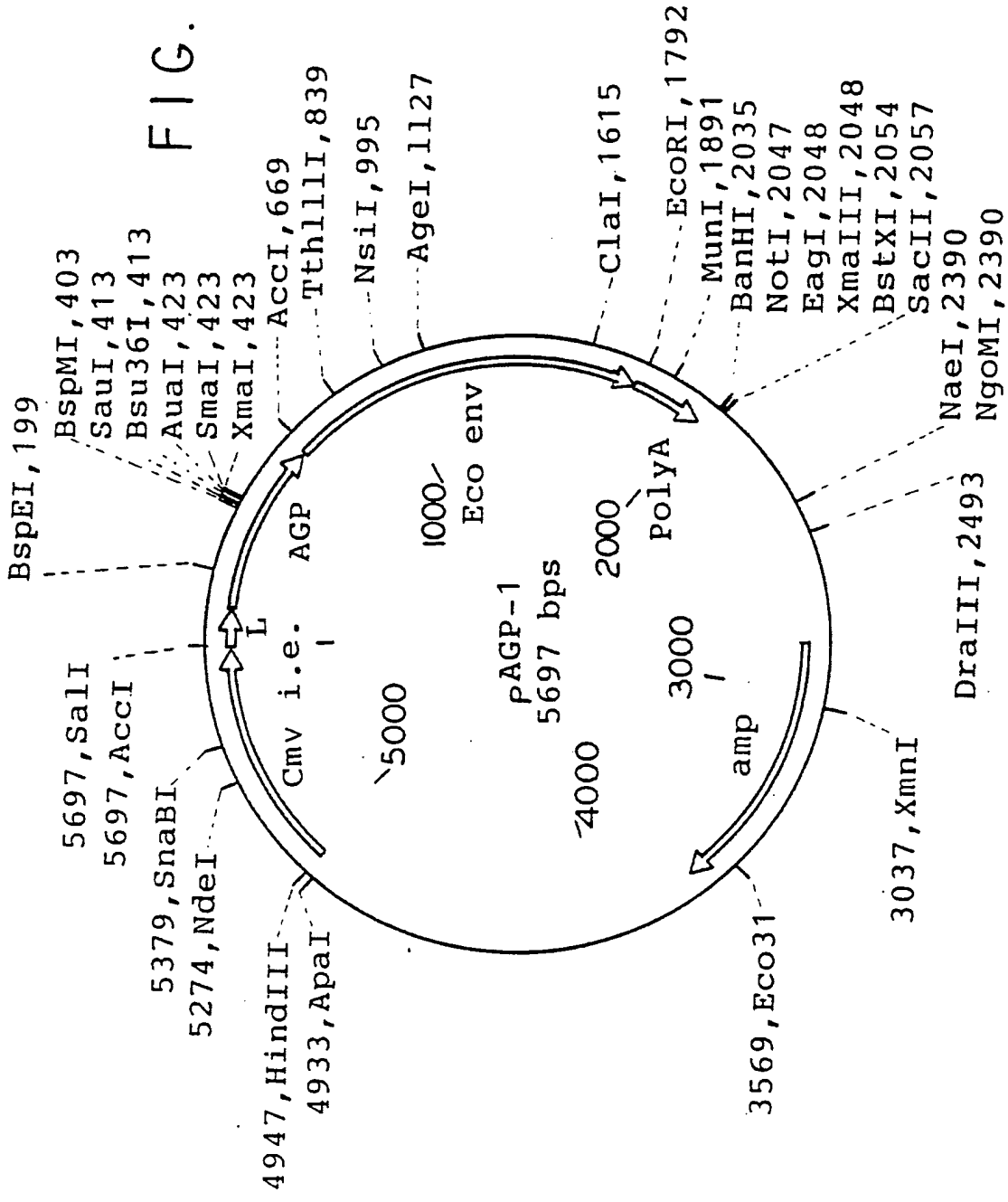
3000
4000
5000
6000

FIG. 2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Eco env
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BspMI,403
SauI,413
Bsu36I,413
AuaI,423
SmaI,423
XmaI,423
AccI,669
TthlllI,839
NsiI,995
AgeI,1127
ClaI,1615
EcoRI,1792
MunI,1891
BanHI,2035
NotI,2047
EagI,2048
XmaIII,2048
BstXI,2054
SacII,2057
NaeI,2390
NgoMI,2390
DraIII,2493
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4933,ApaI
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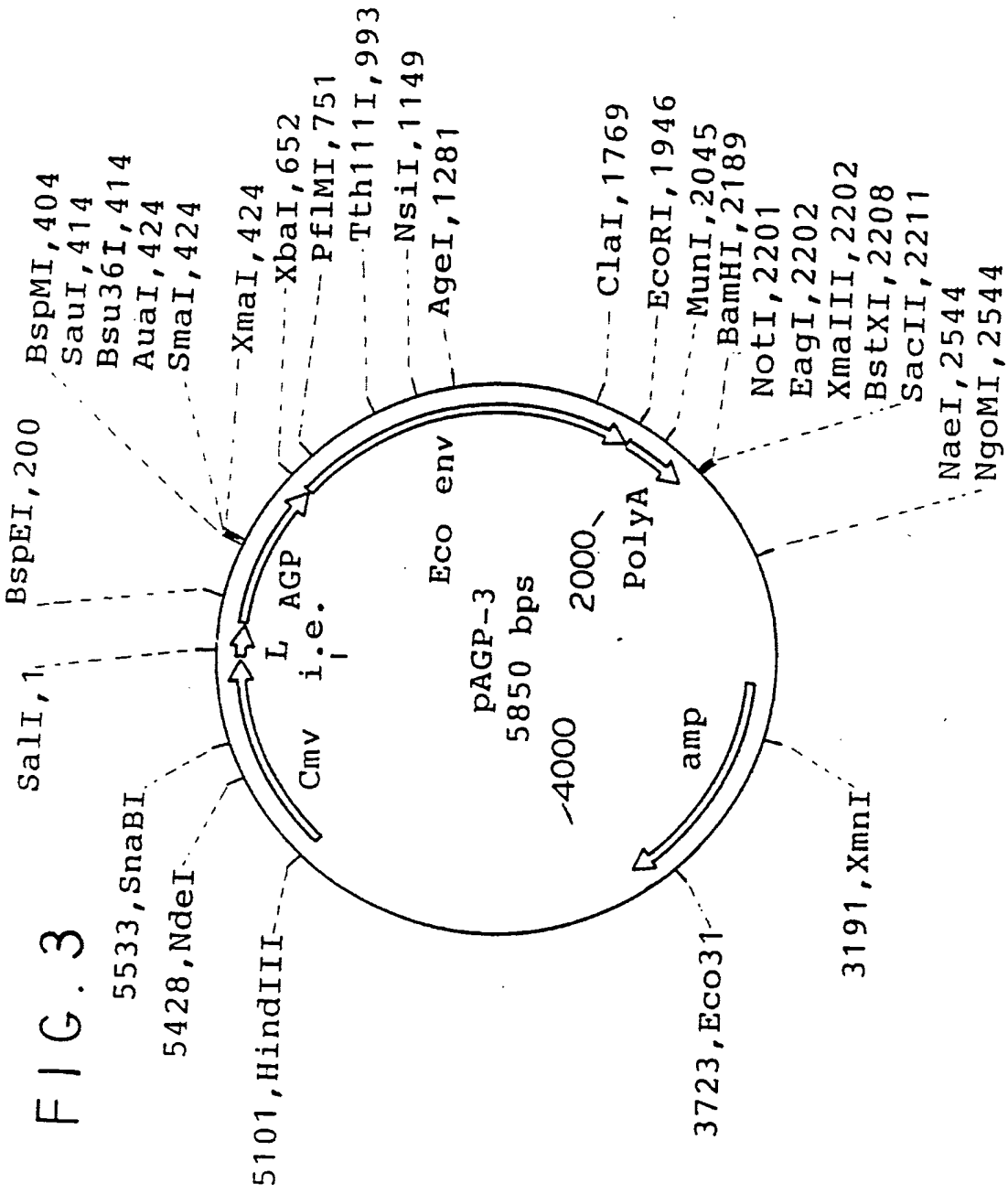
5697,SalI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Bsu36I,414
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XmaI,424
XbaI,652
PflMI,751
Tth111I,993
NsiI,1149
AgeI,1281
ClaI,1769
EcoRI,1946
MunI,2045
BamHI,2189
NotI,2201
EagI,2202
XmaIII,2202
BstXI,2208
SacII,2211
NaeI,2544
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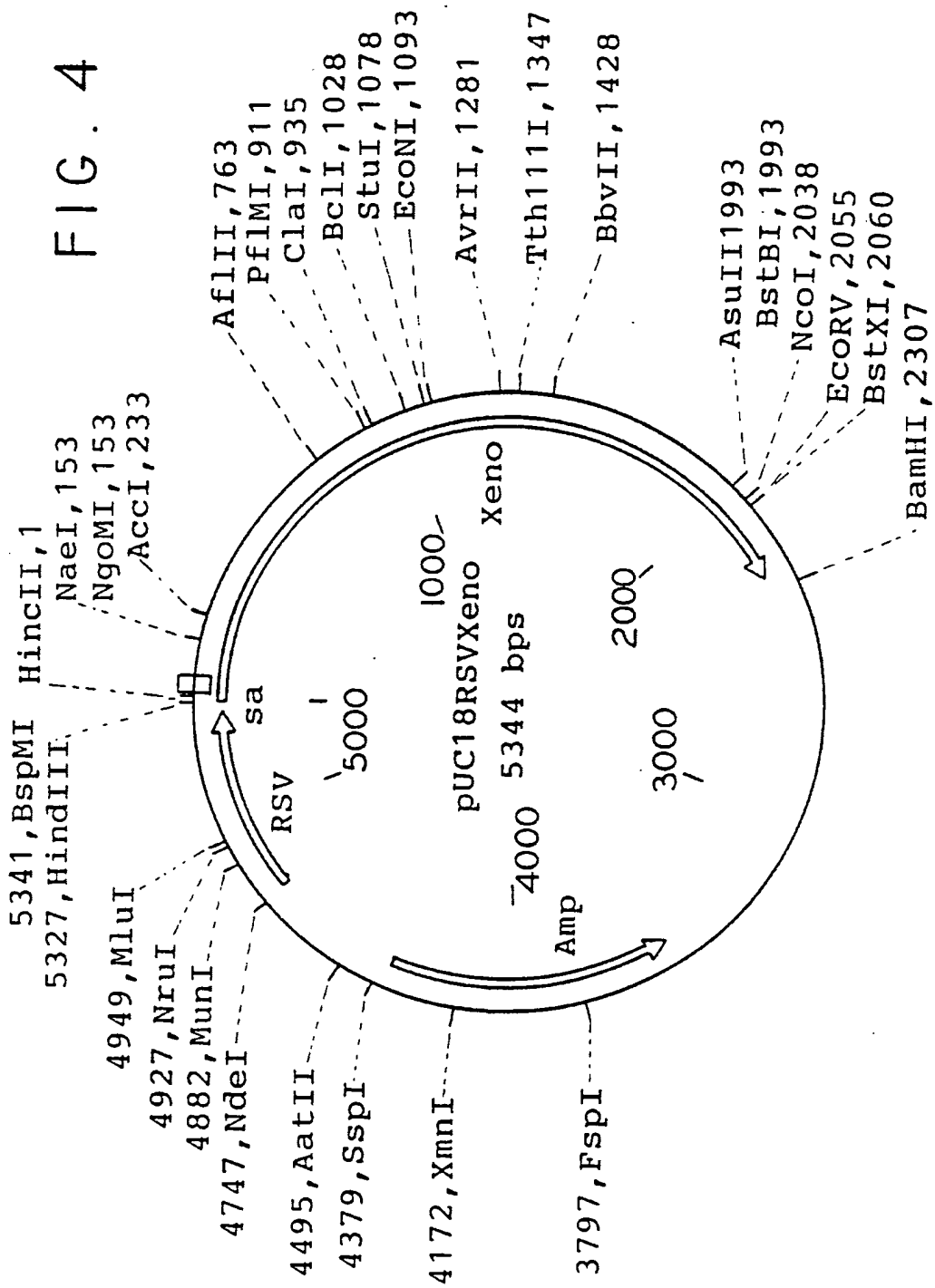
5101,HindIII
5428,NdeI
5533,SnaBI

FIG. 3

FIG. 4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AflII,763
PflMI,911
ClaI,935
BclI,1028
StuI,1078
EcoNI,1093
AvrII,1281
Tth111I,1347
BbvII,1428
AsuII1993
BstBI,1993
NcoI,2038
EcoRV,2055
BstXI,2060
BamHI,2307
5341,BspMI
5327,HindIII
4949,MluI
4927,NruI
4882,MunI
4747,NdeI
4495,AatII
4379,SspI
4172,XmnI
3797,FspI
sa
RSV
Xeno
pUC18RSVXeno
5344 bps
Amp
1000
2000
3000
4000
5000

FIG. 5

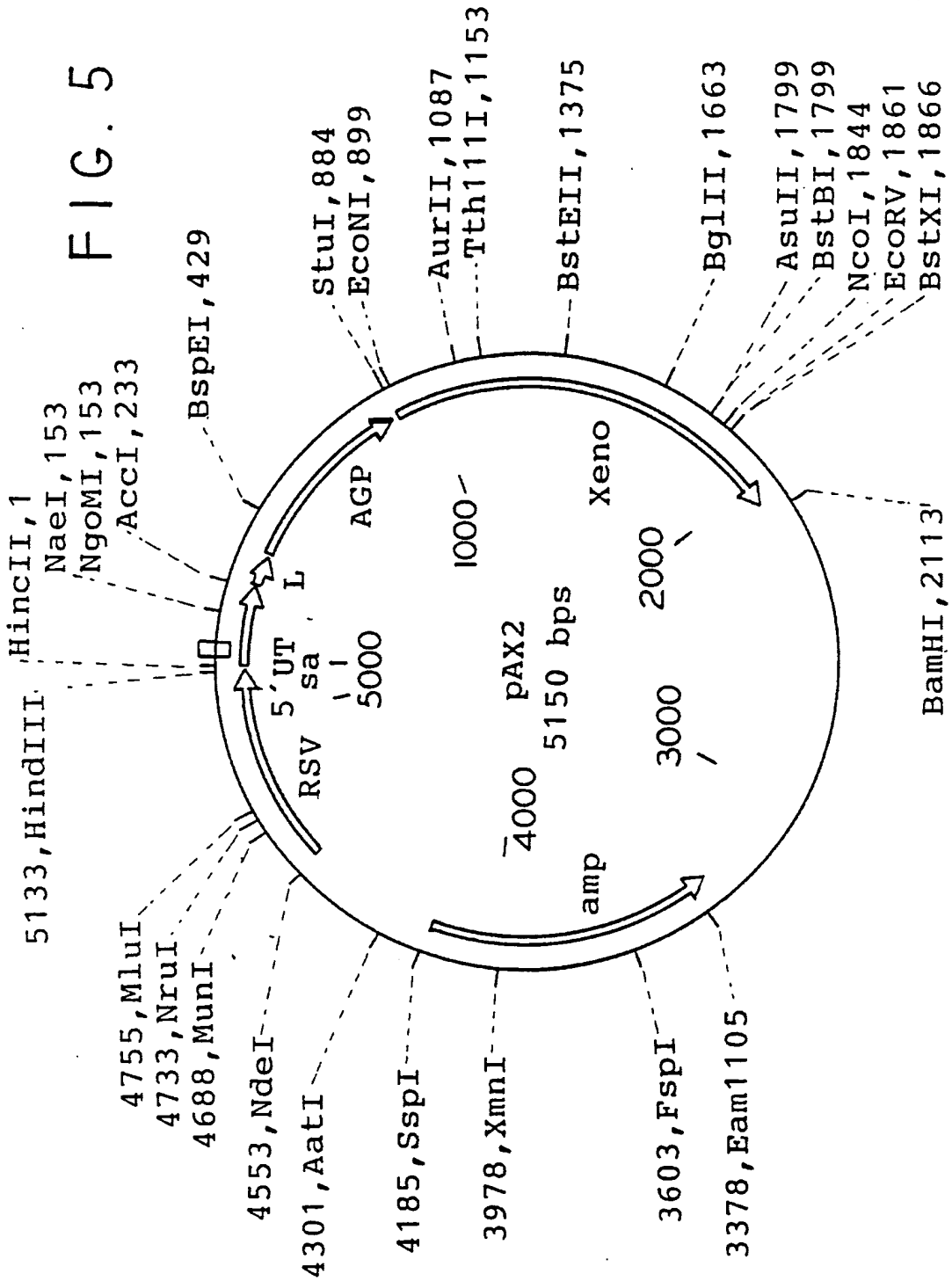
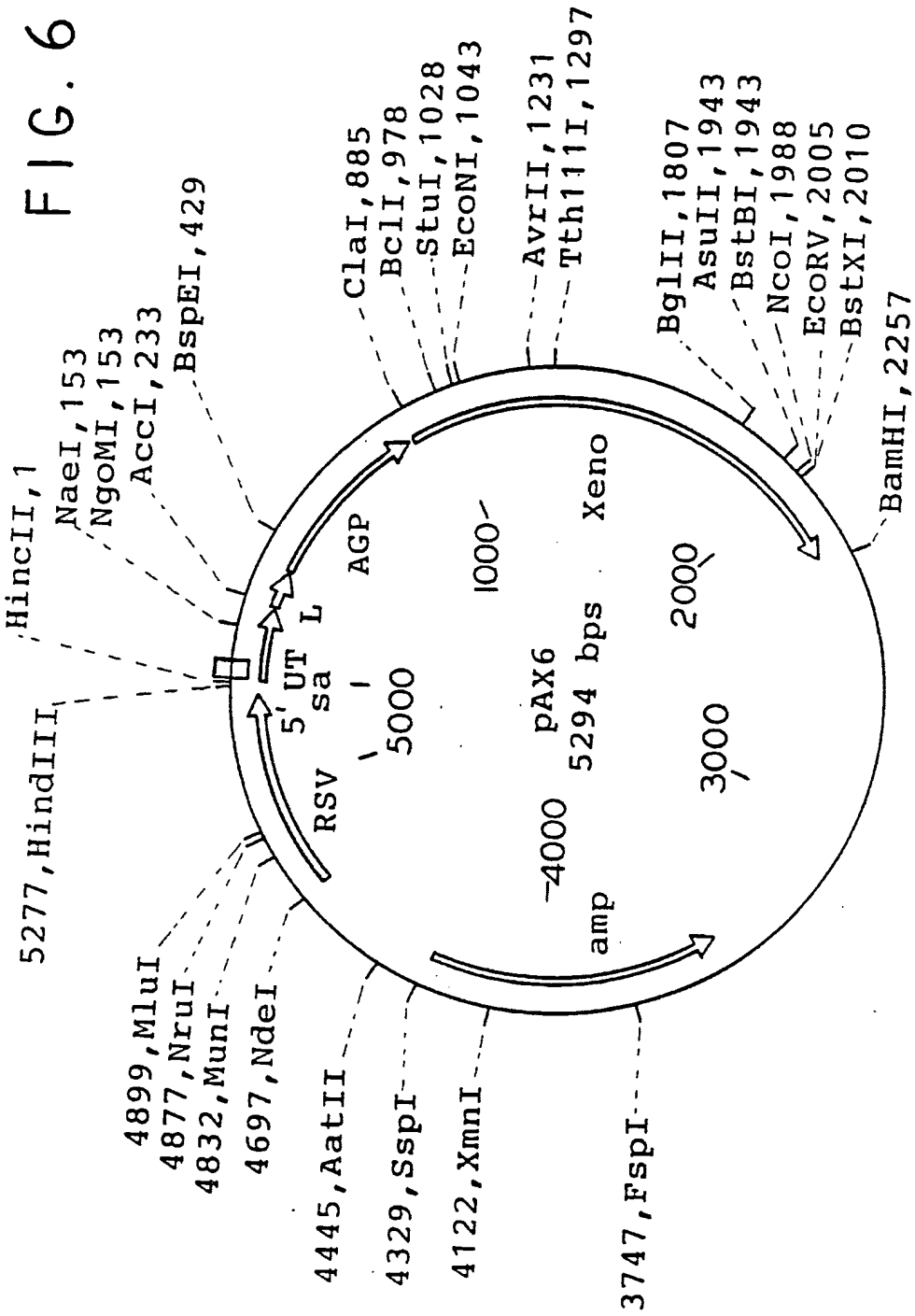

HincII,1
NaeI,153
NgoMI,153
AccI,233
BspEI,429
StuI,884
EcoNI,899
AurII,1087
Tth111I,1153
BstEII,1375
BglII,1663
AsuII,1799
BstBI,1799
NcoI,1844
EcoRV,1861
BstXI,1866
BamHI,2113
Xeno
AGP
L
5'UT
sa
RSV
1000
2000
3000
4000
5000
pAX2
5150 bps
amp
5133,HindIII
4755,MluI
4733,NruI
4688,MunI
4553,NdeI
4301,AatI
4185,SspI
3978,XmnI
3603,FspI
3378,Eam1105

FIG. 6


HincII,1
NaeI,153
NgoMI,153
AccI,233
BspEI,429
ClaI,885
BclI,978
StuI,1028
EcoNI,1043
AvrII,1231
Tth111I,1297
BglII,1807
AsuII,1943
BstBI,1943
NcoI,1988
EcoRV,2005
BstXI,2010
BamHI,2257
5277,HindIII
4899,MluI
4877,NruI
4832,MunI
4697,NdeI
4445,AatII
4329,SspI
4122,XmnI
3747,FspI
5' UTL
sa
AGP
Xeno
RSV
pAX6
5294 bps
amp
1000
2000
3000
4000
5000

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10522

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12P 21/00; C12N 15/00, 15/58, 15/40, 15/48, 15/63, 15/86
US CL :435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Biosis, Biotech, Medicine, Medline
Search Terms: retrovirus, vector, receptor, receptor binding protein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Journal of Virology, Volume 61, No. 5, issued May 1987, M.A. Bender et al., "Evidence that the Packaging Signal of Moloney Murine Leukemia Virus Extends Into the gag Region" pages 1639-1646, See particularly page 1640.	1-14
A	Biotechniques, Volume 7, No. 9, issued 1989, A.D. Miller et al., "Improved Retroviral Vectors for Gene Transfer and Expression" pages 980-990, See particularly page 984.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
05 JANUARY 1994

Date of mailing of the international search report
26 JAN 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/04565

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K47/48

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 29, 15 October 1989, BALTIMORE,USA pages 16985 - 16987 WU ET AL 'TARGETING GENES:DELIVERY AND PERSISTENT EXPRESSION OF A FOREIGN GENE DRIVEN BY MAMMALIAN REGULATORY ELEMENTS IN VIVO' see the whole document ---	1-48
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 264, no. 21, 25 July 1989, BALTIMORE,USA pages 12126 - 12129 KANEDA ET AL 'INTRODUCTION AND EXPRESSION OF THE HUMAN INSULIN GENE IN ADULT RAT LIVER' see the whole document --- -/--	1-48

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 OCTOBER 1992	24. 11. 92
International Searching Authority	**Signature of Authorized Officer**
EUR PEAN PATENT OFFICE	SITCH W.D.C.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 266, no. 6, 25 February 1991, BALTIMORE,USA pages 3361 - 3364 KATO ET AL 'EXPRESSION OF HEPATITIS B VIRUS SURFACE ANTIGEN IN ADULT RAT LIVER' see the whole document	1-48
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES,USA vol. 87, April 1990, WASHINGTON D.C.,USA pages 2652 - 2656 SHALABY ET AL 'EXON SKIPPING DURING SPLICING OF ALBUMIN MRNA PRECURSORS IN NAGASE ANALBUMINEMIC RATS' cited in the application see P.2652,abstract	1-48
A	WO,A,9 012 096 (PURDUE RESEARCH FOUNDATION) 18 October 1990 see page 1, line 25 - page 7, line 4	1-48
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 266, no. 22, 5 August 1991, BALTIMORE,USA pages 14338 - 14342 WU ET AL 'RECEPTOR-MEDIATED GENE DELIVERY IN VIVO' see the whole document	1-3, 6-16, 19-21, 26-28, 31-39, 42-48

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9204565 SA 61035

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 29/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9012096	18-10-90	US-A- 5108921	28-04-92
		AU-A- 5356490	05-11-90
		AU-A- 5437590	05-11-90
		CA-A- 2013580	03-10-90
		CA-A- 2013582	03-10-90
		EP-A- 0466816	22-01-92
		WO-A- 9012095	18-10-90